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(54) Title: STENT WITH SUSTAINED DRUG DELIVERY

(57) Abstract

A mechanical support or stent containing pharmaceutical agents. The stent can be placed in the wall of a blood vessel where it releases pharmaceutical agents to prevent arterial thromboses, platelet aggregation and/or excessive endothelial cell proliferation at the placement site. The stent may also be placed in a blood vessel, bile duct, ureter, or fallopian tube or other duct or vessel, so that it delivers drugs to specific body sites or organs.



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DESCRIPTION

# Stent With Sustained Drug Delivery

This invention relates generally to a mechanical support or stent containing pharmaceutical agents, and a method of using the same. More particularly, this invention relates to a stent containing pharmaceutical agents to be placed in a blood vessel where it preserves luminal dilation and releases agents that prevent arterial thrombosis, platelet aggregation, and/or excessive endothelial cell proliferation at the implant site; or to be placed in a blood vessel, bile duct, ureter, fallopian tube or other duct or vessel where it delivers pharmaceutical agents to specific body sites or organs.

## Background

Despite steady progress in treatment and prevention, atherosclerotic cardiovascular disease remains the most common cause of death in industrialized countries. (AJR 150:1263-1269 (1988)). Although surgical methods of treating atherosclerosis, such as prosthetic replacement of the aorta and cardiac valves and coronary bypass surgery, have resulted in significant medical advancement, a need continues to exist for treatment with less expensive and less invasive techniques.

Percutaneous transluminal angioplasty (PTA), or balloon angioplasty, of peripheral and coronary arteries has proven to be a useful nonsurgical procedure for the treatment of localized occlusive arterial lesions due to atherosclerosis. (Merck Manual, 15th Ed., p. 559). The technique consists of inserting an uninflated balloon-tipped catheter into the affected artery. Dilation of the diseased segment of artery is accomplished by inflating the balloon which pushes the sclerotic lesion outward, thereby enlarging the arterial diameter. The balloon is then deflated and the catheter is withdrawn.

Following PTA, blood flow through the artery is typically significantly improved. Unfortunately, however, although more than 90% of dilations are initially successful, there is a high rate of early failure or later restenosis. About one-third of all patients treated with PTA return for a second or third procedure, thus reducing the long-term benefits of the procedure. (Eur. Heart J. 9:31-37 (1988)).

Some researchers have found most vessels that occluded after PTA revealed disrupted intima and a medial tear that extended to the internal elastic lamina, and that platelet deposition was extensive giving rise to early thrombosis. (Tex. Heart Inst. J. 15(1):12-16 (1988)). Longer balloon inflation times, high doses of calcium-channel blockers, steroids, and other drug regimens have been attempted, but so far have proved unsuccessful in combating this problem. (NEJM 316:701 (1987)).

To increase the long-term benefits of PTA, with the aim of preventing restenosis and sudden closure of diseased arteries after angioplasty, various intravascular prosthetic devices have been developed that can be placed across the freshly-dilated lesion.

Mechanical intraluminal stents have been suggested as an adjunct to PTA in the treatment of atherosclerosis. In 1969, Dotter et al., reported the first non-operative placement of coiled, stainless steel, wire stents in the arteries of dogs. (Invest. Radiol. 4:329-332 (1969). Fourteen years later, several reports on intravascular stents were published. (Radiology 147:261-263 (1983); Radiology 147:259-260 (1983); Radiology 152:659-663 (1984); Radiology 156:69-72 (1985); Radiology 156:73-77 (1985)). And recently Fischell et al. disclosed an invention for a coil spring intravascular stent. (U.S. Patent No. 4,768,507, issued September 6, 1988).

Intravascular stents function by opposing recoil of elastic vascular stenoses after angioplasty has failed.

They are also intended to provide a framework and support for arterial lesions that are likely to dissect after PTA. Although intravascular stents may be quite varied in design, they have been constructed of alloys of nickel and titanium (Id.), tempered stainless steel (Id.), plastic (Radiology 162:276-278 (1987)), or polyester (Tx. Heart Inst. J. 15:12 (1988)), and have three basic mechanisms of action: thermal memory, spring load, and plastic deformation. (AJR, 150:1263-1269 (1988)).

Stents have been used to maintain the patency of many other ducts or vessels as well. Stents placed in the ureter have been described for treatment of obstructions due to benign and malignant lesions. (J. of Urology 130:553-554 (1983)). As a method of nonoperative drainage in the case of obstructive jaundice, stents have been placed in the bile ducts for percutaneous drainage of the biliary system. (Gastrointest. Radiol. 10:394-396) (1985)).

Although most of the previously employed stents exhibited long-term patency of the vessel, failure commonly occurred when excessive endothelial cell growth caused significant narrowing of the lumen. (Radiology, 162:469-472 (1987)). In addition, thrombus formation in small diameter stents has been shown to reduce the lumen diameter and decrease blood flow. (Radiology 102:276-278 (1987)). A need exists therefore, for a stent that retains vessel patency as well as inhibits luminal narrowing.

To date, the placement of intravascular stents in humans has required extensive systemic anticoagulant treatment in an attempt to diminish thrombogenicity of the stents. Sigwart et al report the administration of oral anticoagulants (acenocoumarin) and antiplatelet drugs for at least three months following stent placement. (Eur. Heart J. 9:31-37 (1988)). As with many systemically administered anticoagulants, the chief complication is overdose and the resulting abnormal bleeding which

predisposes to massive hemorrhage if left unchecked. (Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., 1975). Because of this risk, improvements of stent technology are necessary.

Among the other complications encountered with the use of stents in humans were local spasms which occurred immediately after stent placement. To prevent these vasospasms, one researcher reports using nifedipine three times per day for three months. (Eur. Heart J., supra at 32.) Obviously avoidance of the systemic use of these antispasmotics would also be desirable.

Drug therapy now exists that can prolong useful life in persons diagnosed with cancer. Drug development for cancer began with the accidental identification of the antitumor activity of nitrogen mustard, and its success in the treatment of Hodgkin's disease and lymphocytic lymphomas. (Principles of Internal Medicine 9th Ed. p. 1601.) Since the 1950's when it was recognized that a standardized approach to the development of anticancer drugs was needed, many substances have been identified as having antitumor activity. Most of these drugs however, require systemic treatment which destroys cancer cells but also has adverse effects or toxicities on normal cells. A need continues for a method of drug delivery that would destroy cancer cells but not harm normal cells.

Additionally, the conventional methods of drug therapy, including tablets, capsules, slow-release formulations and injectables, all result in typical fluctuations of drug concentrations in the blood and body tissues. If the drug is in tablet or capsule form for example, it dissolves and releases the drug in high concentrations in the stomach; as the drug begins to be absorbed, its concentration in the body rapidly rises to a peak, followed by a decline related to its characteristic metabolism and elimination. With every dose of the drug, concentrations may alternately reach levels that produce adverse side effects and then decline

to values significantly less than therapeutic. As a result, in order to be effective, potent agents destined to treat specific organs must travel through the blood stream in much larger concentrations than those required at the target tissue. (Med. Res. Rev., 1(4):373-386 (1981)). A need exists therefore, for new types of drug delivery methods, to assure an adequate therapeutic effect while reducing or eliminating side effects.

## Summary Of The Invention

The present invention provides a stent with sustained drug release capabilities which is believed to avoid the cited disadvantages of the prior art structures and methods.

Thus it is the objective of the present invention to provide an intravascular stent that preserves vessel patency and inhibits luminal narrowing.

A second objective of the invention is to provide a stent that can be placed in a vessel or duct and deliver a pharmaceutical agent to a specific body site or organ, thereby minimizing the systemic effect of these agents and adverse or toxic effects on other cells.

## Detailed Description Of The Invention

The mechanical support or stent of this invention may be formed from any of the materials employed in the prior art that are non-toxic to the blood and body tissue and otherwise biocompatable. The stent may be in the form of any structure that successfully preserves the luminal diameter of a vessel or duct, and may operate by any mechanism known in the art.

The pharmaceutical agents suitable to be employed in this invention are too numerous to list. The agents may be anticoagulants, antiplatelet substances, antispasmodics or drugs that inhibit excessive endothelial cell growth, or they may be antimicrobial agents, hormones or anticancer drugs, or any combination of these agents, or

any others to accomplish any other localized purpose. The precise coating or impregnating of the stent with the pharmaceutical agent will vary depending on the form and material of the stent, and upon the pharmaceutical agent employed.

In use, the stent is placed into the vessel or duct so that it is in communication with the blood or other body fluid by means described in the art. A preferable means is the catheter insertion method as described by Fischell et al in U.S. Patent No. 4,768,507.

Thereafter, blood or other body fluids will come into contact with the stent which will release a sustained amount of the pharmaceutical agent at the placement site, and/or to specific tissues or organs.

In a preferred embodiment of the invention, an intravascular stent may contain heparin, aspirin, prostacyclin or an analog which when released by the stent, results in inhibition of thrombus formation or excessive endothelial cell growth.

In another embodiment, an intravascular stent may contain antitumor drugs, which, when released, result in antitumor activity.

By constructing a stent according to the above invention, several advantages may be realized. First, placement of the stent within a vessel will release anticoagulants, antiplatelet drugs or drugs that inhibit excessive endothelial cell growth at the placement site, thereby preserving the vessels patency and inhibiting luminal narrowing. Second, placement of a stent containing pharmaceutical agents, will deliver the agents to the placement site and/or to a specific body site or organ, thereby minimizing the systemic effect of these agents and adverse or toxic effects on other cells.

Other and further embodiments of the invention are readily apparent from the above description of the invention, and these embodiments are believed to be within the scope of the invention.

Claims:

1. A stent for placing in a vessel or duct which comprises:

a. a support means that preserves the luminal diameter of said vessel or duct; and

b. said means containing at least one pharmaceutical agent capable of sustained release from the stent.

2. A stent according to claim 1 wherein the vessel or duct is an artery, vein, bile duct, ureter, fallopian tube, or pancreatic duct.

3. A stent according to claim 1 wherein the support means is made of alloys of nickel and titanium, stainless steel, plastic or polyester.

4. A stent according to claim 1 wherein the support means functions to preserve the luminal diameter of a vessel by thermal memory, spring load or plastic deformation.

5. A stent according to claim 1 wherein the pharmaceutical agent is an anticoagulant, antiplatelet substance, antispasmotic, drug that inhibits excessive cell proliferation, antimicrobial agent, hormone, anti-tumor drug, calcium channel blocker or antiarhythmic drug.

6. A method for the sustained release of at least one pharmaceutical agent into a bodily fluid, which comprises:

a. placing a stent containing said pharmaceutical agent(s) into a vessel or duct;

b. said stent being in contact with the fluid in said vessel or duct; and

c. said stent thereby releasing said pharmaceutical agent(s) into said fluid.

7. A method according to claim 6 wherein the pharmaceutical agent is anticoagulant, antiplatelet substance, antispasmotic, drug that inhibits excessive cell proliferation, antimicrobial agent, hormone, antitumor drug, calcium channel blocker or antiarhythmic drug.

8. A method according to claim 6 wherein the bodily fluid is blood, urine or bile.

9. A method according to claim 6 wherein the stent is made of alloys of nickel and titanium, stainless steel, plastic or polyester.

10. A method according to claim 6 wherein the stent functions by thermal memory, spring load or plastic deformation.

11. A method acording to claim 6 wherein the vessel or duct is an artery, vein, bile duct, fallopian tube, or pancreatic duct.

12. A method according to claim 6 wherein the stent is placed into a vessel or duct by catheter insertion.

13. A method for treating atherosclerotic cardiovascular disease comprising:

a. placing a stent containing at least one pharmaceutical agent into a blood vessel;

b. said stent being in contact with the blood in said vessel; and

c. said stent thereby releasing said pharmaceutical agent(s) into said blood and to the placement site.

14. A method according to claim 13 wherein the stent is made of alloys of nickel and titanium, stainless steel, plastic or polyester.

15. A method according to claim 13 wherein the stent functions by thermal memory, spring load or plastic deformation.

16. A method according to claim 13 wherein the pharmaceutical agent is an anticoagulant, antiplatelet drug, antispasmotic, or drug that inhibits excessive endothelial cell proliferation.

17. A method according to claim 13 wherein the blood vessel is a peripheral or coronary artery.

18. A method according to claim 13 wherein the stent is placed into the blood vessel by catheter insertion.

19. A method for treating tumors comprising:

a. placing a stent containing a least one anti-tumor agent into a vessel or duct;

b. said stent being in contact with the fluid in the vessel or duct; and

c. said stent thereby releasing said antitumor agent(s) into said fluid and to said tumor.

20. A method according to claim 19 wherein the stent is made of alloys of nickel and titanium, stainless steel, plastic or polyester.

21. A method according to claim 19 wherein the stent functions by thermal memory, spring load or plastic deformation.

22. A method according to claim 19 wherein the vessel or duct is an artery, vein, bile duct, ureter, fallopian tube, or pancreatic duct.

23. A method according to claim 19 wherein the stent is placed into the vessel or duct by catheter insertion.

24. A method for treating a diseased organ or tissue comprising:

a. placing a stent containing at least one pharmaceutical agent into a vessel or duct;

b. said stent being in contact with the fluid in the vessel or duct; and

c. said stent thereby releasing said pharmaceutical agent(s) into said fluid and to said diseased organ or tissue.

25. A method according to claim 24 wherein the stent is made of alloys of nickel and titanium, stainless steel, plastic or polyester.

26. A method according to claim 24 wherein the stent functions by thermal memory, springload or plastic deformation.

27. A method according to claim 24 wherein the vessel or duct is an artery, vein, bile duct, ureter, fallopian tube, or pancreatic duct.

28. A method according to claim 24 wherein the pharmaceutical agent is an antimicrobial agent, or anti-tumor agent.

29. A method according to claim 24 wherein the stent is placed into the vessel by catheter insertion.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02497

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

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US 604/265, 606/191

## II. FIELDS SEARCHED

Minimum Documentation Searched [4]

| Classification System | Classification Symbols |
|---|---|
| U.S. | 606/191-200<br>604/890.1, 891.1, 892.1, 49.54,55,93,104,265,281,285<br>623/1.11,12,66 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [5]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| X<br>Y | PCT, B WO89/03232 (BUKH MEDITLC) 20 April 1989.<br>See entire document. | 1-16,18-29<br>17 |
| X<br>Y | US, A, 3,948,254 (ZAFFRONI) 06 April 1976.<br>See entire document. | 1-11,24-28<br>12-23,29 |
| X<br>Y | US,A, 3,279,996 (LONG, JR. ET AL.) 18 October 1966.<br>See entire document. | 1-11,13-17,<br>24-28<br>12,18,29 |
| X<br>Y | US, A, 4,321,711 (MANO) 30 March 1982.<br>See entire document. | 1-11,13-16,<br>24-28<br>17 |
| X<br>Y | US, A, 4,642,111 (SAKAMOTO ET AL.) 10 February 1987.<br>See entire document. | 19-22,24-28<br>23,29 |

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
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## (54) Dipyridamole for the treatment of proliferative diseases.

(57) A method of inhibiting cell proliferation in mammals which comprises the local delivery of an inhibitory amount of dipyridamole. Inhibiting cell proliferation is useful for the treatment of proliferaive diseases such as vascular restonesis, scleroderma, psoriasis, and rheumatoid arthritis. This method includes the local delivery of dipyridamole to the proliferative site by various techniques including local delivery catheters, site specific carriers, implants, direct injection, or direct application.

EP 0 543 653 A1

This invention relates to the local delivery of dipyridamole for the treatment of proliferative diseases.

Proliferative diseases such as vascular restenosis, scleroderma, psoriasis, and rheumatoid arthritis share the fundamental mechanism of excessive proliferation of cells in a specific tissue or organ. In each of these diseases, the excessive proliferation of cells contributes significantly to the pathogenesis of the disease.

For example, vascular restenosis is characterized by the reocclusion of coronary arteries following percutaneous transluminal coronary angioplasty (PTCA), atherectomy, laser angioplasty and arterial bypass graft surgery. The reocclusion of coronary arteries is caused in part by the excessive proliferation of vascular smooth muscle cells. See "Intimal Proliferation of Smooth Muscle Cells as an Explanation for Recurrent Coronary Artery Stenosis after Percutaneous Transluminal Coronary Angioplasty," Austin et al., Journal of the American college of Cardiology 6: 369-375 (Aug. 1985).

Vascular restenosis remains a major long term complication following surgical intervention of blocked arteries by percutaneous transluminal coronary angioplasty (PTCA), atherectomy, laser angioplasty and arterial bypass graft surgery. In about 35% of the patients who undergo PTCA, reocclusion occurs within three to six months after the procedure. The current strategies for treating vascular restenosis include mechanical intervention by devices such as stents or pharmacologic therapies including heparin, low molecular weight heparin, coumarin, aspirin, fish oil, calcium antagonist, steroids, and prostacyclin. These strategies have failed to curb the reocclusion rate and have been ineffective for the treatment and prevention of vascular restenosis. See "Prevention of Restenosis after Percutaneous Transluminal Coronary Angioplasty: The Search for a 'Magic Bullet'," Hermans et al., American Heart Journal 122: 171-187 (July 1991).

The excessive proliferation of fibroblast and mesenchymal cells is associated with rheumatoid arthritis and psoriasis. The inflammatory process that is characteristic of rheumatoid arthritis results in the release of growth factors that induce active proliferation of mesenchymal cells. This proliferation is associated with the production of excessive amounts of enzymes capable of destroying the connective tissues that comprise the joint. Pharmacologic agents that inhibit the proliferative response would be effective in repressing some of the destructive potential of rheumatoid arthritis. See "Recent Insights into the Pathogenesis of the Proliferative Lesion of Rheumatoid Arthritis," Harris, Arthritis and Rheumatism 19: 68-72 (January-February 1976).

Scleroderma (systemic sclerosis) is a multisystem disease affecting primarily the vascular, cutaneous, musculoskeletal, gastrointestinal, pulmonary, cardiac, and renal systems. The apparent diffuse clinical features of systemic sclerosis are thought to be linked by a distinctive vascular lesion in the various target organs. This vascular lesion has inflammatory, proliferative, and indurative phases and is clearly related to the proliferation of the fibroblast and cells capable of fibroblast activity. Controlling this mechanism of fibroblastic activation and proliferation may be useful in treating or preventing systemic sclerosis. See "Pathogenesis of Systemic Sclerosis: A Vascular Hypothesis," Campbell et al., Seminars in Arthritis and Rheumatism 4: 351-368 (May, 1975).

In the pathogenesis of proliferative diseases, excessive cell proliferation occurs as a result of the presence of various growth factors and cytokines such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and interleukin-1 (IL-1). For example, growth factors produced by cellular constituents in the blood and the damaged arterial vessel wall mediate the proliferation of smooth muscle cells in vascular restenosis. A novel method of administering dipyridamole to inhibit cellular proliferation caused by various growth factors is therefore useful for the treatment of proliferative diseases such as psoriasis, rheumatoid arthritis, scleroderma, and vascular restenosis. The American Journal of Medicine70: 1231-1236 (June 1981).

Dipyridamole is commonly prescribed as an antiplatelet or phosphodiesterase inhibitor. It has been studied independently or in conjunction with aspirin and/or prostacyclin for the treatment of vascular restenosis. The results of these studies have demonstrated that dipyridamole, when systemically administered, is ineffective in treating or preventing vascular restenosis in patients. Hermans et al., American Heart Journal 122: 171-187 (July, 1991); Harker et al., Arteriosclerosis 10: 828a (September-October, 1990); and FitzGerald, The New England Journal of Medicine 316:1247-57 (May, 1987). The use of dipyridamole for the treatment of restenosis has been ineffective in these studies due to the systemic method of administration. Serum reduces the effectiveness of systemically administered dipyridamole as an inhibitor of cell proliferation.

Only upon the observation of the effects of serum on systemically administered dipyridamole did it finally become possible to discover the use of dipyridamole as an antiproliferative agent. The invention discloses the local delivery of dipyridamole as a method of inhibiting cell proliferation and is useful for the treatment of proliferative diseases such as restenosis, scleroderma, psoriasis, and rheumatoid arthritis.

This invention provides a method of inhibiting cell proliferation in mammals which comprises the local delivery of an inhibitory amount of dipyridamole.

Dipyridamole is a well known compound used extensively as a coronary vasodilator. The Merck Index Tenth Edition: 3366 (1983). Its chemical name is 2,6-bis (diethanol-amino)-4,8-dipiperidinopyrimido[5,4-d]pyrimidine.

Its preparation is disclosed in British patent 807,826 (1959 to Thomae).

The local delivery of inhibitory amount of dipyridamole for the treatment of cell proliferation can be by a variety of techniques which administer the dipyridamole at or near the proliferative site. Examples of local delivery techniques are not intended to be limiting but to be illustrative of the techniques available. Examples include local delivery catheters, site specific carriers, implants, direct injection, or direct applications.

Local delivery by a catheter allows the administration of a pharmaceutical agent directly to the proliferative lesion. Examples of local delivery using a balloon catheter are described in EPO 383 492 A2 and U.S. Patent 4,636,195 (Wolinsky, January 13, 1987).

Local delivery by an implant describes the surgical placement of a matrix that contains the pharmaceutical agent into the proliferative lesion. The implanted matrix releases the pharmaceutical agent by diffusion, chemical reaction, or solvent activators. Langer, Science 249: 1527-1533 (September, 1990). An example of local delivery by an implant is the use of a stent. Stents are designed to mechanically prevent the collapse and reocclusion of the coronary arteries. Incorporating a pharmaceutical agent into the stent delivers the drug directly to the proliferative site. Local delivery by this technique is described in kohn, Pharmaceutical Technology (October, 1990). A second example is a delivery system in which a polymer that contains the pharmaceutical agent is injected into the lesion in liquid form. The polymer then cures to form the implant in situ. This technique is described in PCT WO 90/03768 (Donn, April 19, 1990). Another example is the delivery of a pharmaceutical agent by polymeric endoluminal sealing. This technique uses a catheter to apply a polymeric implant to the interior surface of the lumen. The pharmaceutical agent incorporated into the biodegradable polar implant is thereby released at the surgical site. It is described in PCT WO 90/01969 (Schindler, August 23, 1989). A final example of local delivery by an implant is by direct injection of vesicles or microparticulates into the proliferative site. These microparticulates may be composed of substances such as proteins, lipids, carbohydrates or synthetic polymers. These microparticulates have the pharmaceutical agent incorporated throughout the microparticle or over the microparticle as a coating. Delivery systems incorporating microparticulates are described in Lange, Science 249: 1527-1533 (September, 1990) and Mathiowitz, et al., J. App. Poly. Sci., 26:809 (1981).

Local delivery by site specific carriers describes attaching the pharmaceutical agent to a carrier which will direct or link the drug to the proliferative cells. Examples of this delivery technique includes the use of carriers such as a protein ligand or a monoclonal antibody or a membrane anchored linker. Lange, Science 249: 1527-1533 (September, 1990); Langworth, Genetic Engineering News (September, 1990).

Local delivery by direct application includes the use of topical applications. An example of a local delivery by direct application is applying the pharmaceutical agent directly to the arterial bypass graft during the surgical procedure.

Local delivery by direct injection describes injecting fine particles of dipyridamole suspended in an inert carrier such as sterile saline solution directly into the proliferative site.

The dosage of dipyridamole required to produce the therapeutic effect is dependent upon the method of administration and the particular circumstances of the patient. A therapeutic dosage of dipyridamole is an amount sufficient to inhibit the proliferation of cells. The preferred dosage range is defined to be about 1 µg/day to about 100,000 µg/day delivered at or near the proliferative site.

The term "treatment" includes the administration of a compound of present invention to prevent the onset of the symptoms, alleviating the symptoms, or eliminating the disease, condition, or disorder.

Microparticles for use in conjunction with local delivery catheter can be prepared using the following ingredients: biological surfactant (0.1-3 % by volume), dextrose (2 - 5 % by volume), dipyridamole (milled to 2-10 micron particles, 0.1-10 mg/ml), and water.

The above ingredients are mixed and injected into the proliferative lesion. The following example of a formulation for the local delivery of dipyridamole is illustrative only and are not intended to limit the scope of the invention in any way.

Microparticles were prepared using the ingredients below:

| | Quantity |
|---|---|
| polyoxyethylenesorbitan | 2 % (by volume) |
| dextrose | 5 % (by volume) |
| dipyridamole (milled) | 250 mg |
| water | 50 ml |

The microcapsules are injected into the proliferative lesion using a catheter.

A critical aspect of the present invention provides for the local delivery of dipyridamole to prevent cell proliferation. Cellular proliferation may be induced by cytokines such as interleukin-1 (IL-1) or multiple growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and/or fibroblast growth factor (FGF).

Previous attempts to use dipyridamole for the treatment of restenosis have been ineffective due to the systemic method of administration. Systemic administration includes delivery techniques that introduce the pharmaceutical agent to the entire organism. Examples of systemic delivery include oral and intravenous administration.

Serum reduces the effectiveness of systemically administered dipyridamole as an inhibitor of cell proliferation. The effect of serum on the antiproliferative activity of dipyridamole was demonstrated as follows: Smooth muscle cells from rabbit aorta (derived by explant method as described in Ross, Journal of Cell Biology 50:172 (1971)) were seeded in 96 well tissue culture plates in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY), 2mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Growth arrested, confluent cultures in 96 well microtiter plates were incubated in medium containing 1%, 5% or 20 % serum (Hyclone Laboratories, Logan, Utah), 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 40 ng/ml PDGF (Genzyme, Cambridge, Ma), 1 µci/ml $^3$H thymidine (DuPont, Boston, MA) and indicated concentrations of dipyridamole (Sigma Chemical, St. Louis, MO). Cells were incubated at 37°C for 24 hours under 5% $CO_2$/95% air. At the end of 24 hours, cells were fixed in methanol and DNA synthesis was determined by $^3$H thymidine incorporation as described in Bonin et al., Exp. Cell Res. 181, 475-482 (1989). The following table demonstrates that increasing concentrations of serum attenuate the growth inhibitory effects of dipyridamole.

Table 1

Effect of serum concentration on inhibition of DNA synthesis by dipyridamole.

| Dipyridamole (µg/ml) | % Inhibition of DNA Synthesis | | |
|---|---|---|---|
| | 1% Serum | 5% Serum | 20% Serum |
| 0 | 0 | 0 | 0 |
| 0.04 | 59 | 52 | 6 |
| 0.08 | 70 | 53 | 0 |
| 0.15 | 79 | 60 | 33 |
| 0.3 | 80 | 71 | 38 |
| 0.6 | 86 | 80 | 60 |
| 1.2 | 90 | 87 | 74 |
| 2.5 | 93 | 92 | 77 |

The effect of systemically administered dipyridamole upon cell proliferation was demonstrated as follows:

Balloon injury to the left common carotid arteries of male Sprague-Dawley rats (350-400g) was accomplished by three passes of an inflated 2F Fogarty balloon catheter (Baxter Healthcare, McGaw Park, IL) as described by Clowes et al., Lab Invest. 49: 208-215 (1983). Animals were anesthetized with ketamine (80 mg/kg, intramuscular, Aveco, Ft. Dodge, IA) and Rompun (16 mg/kg, intramuscular, Mobay Corp., Shawnee, KA). Entry of the balloon catheter to the left common carotid artery was made via a nick in the external carotid artery, which was tied off at the end of the surgical procedure. Dipyridamole was systemically administered for two weeks (.03 and .10% wt/wt, as an admixture in the diet, equivalent to approximately 30 and 100 mg/kg/day, respectively). No significant effect upon intimal thickening in the balloon-injured rat carotid arteries was observed as demonstrated in Table 2.

Table 2

| Effect of Systemic Administration of Dipyridamole Upon Intimal Thickening | |
|---|---|
| **Systemic Administration of Dipyridamole (% in diet, wt/wt)** | **Area of Intimal Thickening mm², (% of control)** |
| | |
| 0.00 | 0.120 ± 0.014 (100) |
| | |
| 0.03 | 0.116 ± 0.017 (97.6) |
| | |
| 0.10 | 0.109 ± 0.014 (90.8) |

The effect of the present invention to control the cellular proliferation and intimal thickening by the local delivery of dipyridamole has been demonstrated by in vivo studies. The following examples illustrate the present invention and are not intended to limit the same.

Example 1

Balloon Injury of Carotid Arteries

Balloon injury to the left common carotid arteries of male Sprague-Dawley rats (350-400g) was accomplished by three passes of an inflated 2F Fogarty balloon catheter as described by Clowes et al., Lab Invest. 49: 208-215 (1983). Animals were anesthetized with Ketamine (80 mg/kg, intramuscular) and Rompun (16 mg/kg, intramuscular). Entry of the balloon catheter to the left common carotid artery was made via a nick in the external carotid artery, which was tied off at the end of the surgical procedure. Continuous local delivery of dipyridamole was accomplished by means of a minosmotic pump-implanted subcutaneously in the back of the rat. Pumps were primed before surgery and implanted immediately following balloon injury. Dosing solutions were delivered to the adventitial (exterior) space surrounding the injured carotid artery via a micro-renathane catheter (MRE-40, Baxter Healthcare, Santa Ana, CA) at a rate of 5 µl per hour. The catheter is sutured in place with two ligatures (4-0 silk) to the left external carotid artery.

Fourteen days post surgery, animals were anesthetized (vide supra) and perfused through the abdominal aorta in a retrograde manner at physiological pressure with a zinc formalin fixative (Anatech LTD., Battle Creek, MI). Middle sections (5 mm) of the carotids were removed from the animals, processed, and embedded in paraffin. Three adjacent cross sections (5 µm thick) of each vessel were cut, stained with hematoxylin and eosin, and cross-sectional intimal areas quantitated with an image analyzer (Quantimet 970, Cambridge Inst., Cambridge, UK).

The difference between intimal areas of drug-treated vs. control groups were analyzed for statistical significance using Student's t-test as described in Tallarida et al., Manual of Pharmacologic Calculations with Computer Programs, Springer-Verlag, New York, 1981, p. 51. P values less than 0.05 were taken to indicate statistical significance. The results are demonstrated in Table 3.

Table 3

Effect of Local Administration of Dipyridamole Upon Intimal Thickening

| Local, Adventitial Administration of Dipyridamole (µg/day) | Area of Intimal Thickening $mm^2$, (% of control) |
|---|---|
| 0 (Vehicle) | 0.129 ± 0.013 (100) |
| 600 | 0.087 ± 0.011 (67.4)* |

* P<0.05 vs. corresponding control group (i.e., absence of dipyridamole)

Example 2

Dipyridamole also inhibits proliferation of cells of mesenchyme origin. Inhibition of fibroblast growth by dipyridamole is demonstrated as follows: 20,000 Balb/c3T3 fibroblasts (American Tissue Culture Type, CCL-163) were plated in 12 well tissue culture plates in 3 ml DMEM containing 5% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin, and were incubated for 18-24 hours. Cells were then transferred to above medium containing indicated concentrations of dipyridamole. After three days cell growth was determined by counting using a ZM Coulter counter (Coulter Diagnostic, Inc.).

Table 4

| Inhibition of Fibroblast Growth by Dipyridamole | |
|---|---|
| Dipyridamole (µg/ml) | % Inhibition of Cell Growth |
| 0.0 | 0.0 |
| 1.0 | 0 |
| 5.0 | 55 |
| 10 | 72 |
| 20 | 86 |
| 40 | 95 |

Example 3

Dipyridamole inhibits smooth muscle cell proliferation induced by multiple growth factors. Smooth muscle cells from rabbit aorta (derived by explant method) were seeded in 96 well tissue culture plates in DMEM containing 10% fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Growth arrested, confluent cultures in 96 well microtiter plates were incubated in medium containing 1% platelet poor plasma, 2 mM L-glutanime, 100 U/ml penicillin, 100 µg/ml streptomycin, 20 ng/ml PDGF, 3ng/ml EGF (Genzyme), 3 ng/ml FGF (Genzyme), 1 µci/ml $^3$H thymidine and indicated concentrations of dipyridamole. Cells were incubated at 37°C for 24 hours under 5% $CO_2$/95% air. At the end of 24 hours, cells were fixed in methanol. DNA synthesis was determined by $^3$H thymidine incorporation as previously described. The results in Table show that dipyridamole inhibits cell proliferation induced by PDGF, FGF, and EGF.

Table 5

| Inhibition of DNA Synthesis induced by PDGF, FGF or EGF by dipyridamole. | | | |
|---|---|---|---|
| Dipyridamole (µg/ml) | % Inhibition of DNA Synthesis | | |
| | FGF | EGF | PDGF |
| 0 | 0 | 0 | 0 |
| 0.08 | 67 | 68 | 64 |
| 0.15 | 74 | 76 | 71 |
| 0.32 | 80 | 85 | 82 |
| 0.64 | 87 | 90 | 89 |
| 1.2 | 92 | 94 | 93 |
| 2.5 | 93 | 96 | 96 |

## Claims

1. The use of dipyridamole, in the preparation of a formulation adapted for the local delivery of an effective amount of dipyridamole directly to proliferative cells.
2. The use of dipyridamole, in the preparation of a formulation adapted for the local delivery of an effective amount of dipyridamole directly to proliferative vascular smooth muscle cells.
3. The use of Claim 2 wherein the formulation is adapted for use in conjunction with a local delivery catheter.
4. The use of Claim 2 wherein the formulation is adapted for use in conjunction with a site specific carrier.
5. The use of Claim 2 wherein the formulation is adapted for use in conjunction with an implant.

6. The use of Claim 2 wherein the formulation is adapted for use in conjunction with a membrane anchored linker.

7. The use of Claim 2 wherein the formulation is adapted for use in conjunction with a direct injection.

European Patent Office

# EUROPEAN SEARCH REPORT

Application Number
EP 92 31 0577



## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| X | ARTERIOSCLEROSIS<br>vol. 7, no. 2, March 1987,<br>pages 152 - 158<br>TAKEHARA K. ET AL 'DIPYRIDAMOLE DECREASES PLATELET-DERIVED GROWTH FACTOR LEVELS IN HUMAN SERUM.'<br>* abstract *<br>* Discussion *<br>* page 157, column 1, line 29 - page 157, column 2, line 37 *<br>--- | 1 | A61K31/505 |
| X | THE CANADIAN JOURNAL OF CARDIOLOGY<br>vol. 4, no. 1, January 1988,<br>pages 56 - 59<br>LANDYMORE R.W. ET AL 'CORRELATION BETWEEN THE EFFECTS OF ASPIRIN AND DIPYRIDAMOLE ON PLATELET FUNCTION AND PREVENTION OF INTIMAL HYPERPLASIA IN AUTOLOGOUS VEIN GRAFTS.'<br>* abstract *<br>--- | 1 | |
| X | EXPERIMENTAL AND MOLECULAR PATHOLOGY<br>vol. 48, no. 1, February 1988,<br>pages 116 - 134<br>INGERMANN-WOJENSKI C.M. ET AL 'MODEL SYSTEM TO STUDY INTERACTION OF PLATELETS WITH DAMAGED ARTERIAL WALL'<br>* abstract *<br>* page 119, line 46 - page 120, line 1 *<br>---<br>-/-- | 1 | TECHNICAL FIELDS SEARCHED (Int. Cl.5)<br>A61K |

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 05 FEBRUARY 1993 | MAIR J. |

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

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Application Number

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## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| X | ARTERY<br>vol. 15, no. 4, 1988,<br>pages 225 - 233<br>LINDBLAD B. ET AL 'EFFECT OF ANTICOAGULANT AND ANTIPLATELET DRUGS ON IN VITRO SMOOTH MUSCLE PROLIFERATION'<br>* abstract *<br>* page 229table II *<br>* page 229, line 40 - page 230, line 1 *<br>* page 231, line 11 - page 231, line 14 *<br>--- | 1 | |
| X | ANNALS OF SURGERY<br>vol. 195, no. 3, March 1982,<br>pages 328 - 339<br>HAGEN P.O. ET AL 'ANTIPLATELET THERAPY REDUCES AORTIC INTIMAL HYPERPLASIA DISTAL TO SMALL DIAMETER VASCULAR PROSTHESES (PTFE) IN NONHUMAN PRIMATES'<br>* abstract *<br>--- | 1 | |
| D,A | WO-A-9 001 969 (SLEPIAN M.J. ET AL)<br>8 March 1990<br>* abstract *<br>* page 13, line 21 - page 14, line 5 *<br>--- | 2-5,7<br>2-5,7 | TECHNICAL FIELDS SEARCHED (Int. Cl.5) |
| D,A | WO-A-8 303 356 (WOLINSKY,H.)<br>13 October 1983<br>* abstract *<br>--- | 2-4,7 | |
| D,A | WO-A-9 003 768 (SOUTHERN RESEARCH INSTITUTE)<br>19 April 1990<br>* abstract *<br>---<br>-/-- | 3,5,7 | |

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 05 FEBRUARY 1993 | MAIR J. |

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# EUROPEAN SEARCH REPORT

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## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| D,A | SCIENCE<br>vol. 249, September 1990,<br>pages 1527 - 1533<br>LANGER R. ET AL 'NEW METHODS OF DRUG DELIVERY'<br>* The Whole Document * | 2-7 | |

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 05 FEBRUARY 1993 | MAIR J. |

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## (54) Method of treating hyperproliferative vascular disease using rapamycin, eventually in combination with mycophenolic acid.

(57) This invention provides a method of preventing or treating hyperpoliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

EP 0 551 182 A1

## BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease caused by a partial blockage of the blood vessels that supply the heart with nutrients. More severe blockage of blood vessels in such individuals often leads to hypertension, ischemic injury, stroke, or myocardial infarction. Typically vascular occlusion is preceded by vascular stenosis resulting from intimal smooth muscle cell hyperplasia. The underlying cause of the intimal smooth muscle cell hyperplasia is vascular smooth muscle injury and disruption of the integrity of the endothelial lining. The overall disease process can be termed a hyperproliferative vascular disease because of the etiology of the disease process. Intimal thickening following arterial injury can be divided into three sequential steps: 1 ) initiation of smooth muscle cell proliferation following vascular injury, 2) smooth muscle cell migration to the intima, and 3) further proliferation of smooth muscle cells in the intima with deposition of matrix. Investigations of the pathogenesis of intimal thickening have shown that, following arterial injury, platelets, endothelial cells, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and cytokines that result in the smooth muscle cell proliferation and migration. T-cells and macrophages also migrate into the neointima. [Haudenschild, C., Lab. Invest. 41: 407 (1979); Clowes, A., Circ. Res. 56: 139 (1985); Clowes, A., J, Cardiovas. Pharm. 14 (Suppl. 6): S12 (1989); Manderson, J., Arterio. 9: 289 (1989); Forrester, J., J. Am. Coll. Cardiol. 17: 758 (1991)]. This cascade of events is not limited to arterial injury, but also occurs following injury to veins and arterioles.

Vascular injury causing intimal thickening can be broadly categorized as being either biologically or mechanically induced. Artherosclerosis is one of the most commonly occurring forms of biologically mediated vascular injury leading to stenosis. The migration and proliferation of vascular smooth muscle plays a crucial role in the pathogenisis of artherosclerosis. Artherosclerotic lesions include massive accumulation of lipid laden "foam cells" derived from monocyte/macrophage and smooth muscle cells. Formation of "foam cell" regions is associated with a breech of endothelial integrity and basal lamina destruction. Triggered by these events, restenosis is produced by a rapid and selective proliferation of vascular smooth muscle cells with increased new basal lamina (extracellular matrix) formation and results in eventual blocking of arterial pathways. [Davies, P.F., Artherosclerosis Lab. Invest. 55: 5 (1986)].

Mechanical injuries leading to intimal thickening result following balloon angioplasty, vascular surgery, transplantation surgery, and other similar invasive processes that disrupt vascular integrity. Intimal thickening following balloon catheter injury has been studied in animals as a model for arterial restenosis that occurs in human patients following balloon angioplasty. Clowes, Ferns, Reidy and others have shown that deendothelilization with an intraarterial catheter that dilates an artery injures the innermost layers of medial smooth muscle and may even kill some of the innermost cells. [Schwartz, S.M., Human Pathology 18: 240 (1987); Fingerle, J., Ateriosclerosis 10: 1082 (1990)] Injury is followed by a proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae in the internal elastic lamina and proliferate to form a neointimal lesion.

Vascular stenosis can be detected and evaluated using angiographic or sonographic imaging techniques [Evans, R.G., JAMA 265: 2382 (1991)] and is often treated by percutaneous transluminal coronary angioplasty (balloon catheterization). Within a few months following angioplasty, however, the blood flow is reduced in approximately 30-40 percent of these patients as a result of restenosis caused by a response to mechanical vascular injury suffered during the angioplasty procedure, as described above. [Pepine, C., Circulation 81: 1753 (1990); Hardoff, R., J. Am. Coll. Cardiol. 15 1486 (1990)].

In an attempt to prevent restenosis or reduce intimal smooth muscle cell proliferation following angioplasty, numerous pharmaceutical agents have been employed clinically, concurrent with or following angioplasty. Most pharmaceutical agents employed in an attempt to prevent or reduce the extent of restenosis have been unsuccessful. The following list identifies several of the agents for which favorable clinical results have been reported: lovastatin [Sahni, R., Circulation 80 (Suppl.) 65 (1989); Gellman, J., J. Am. Coll. Cardiol. 17: 251 (1991)]; thromboxane $A_2$ synthetase inhibitors such as DP- 1904 [Yabe, Y., Circulation 80 (Suppl.) 260 ( 1989)]; eicosapentanoic acid [Nye, E., Aust. N.Z. J. Med. 20: 549 (1990)]; ciprostene (a prostacyclin analog) [Demke, D., Brit. J. Haematol 76 (Suppl.): 20 (1990); Darius, H., Eur. Heart J. 12 (Suppl.): 26 (1991)]; trapidil (a platelet derived growth factor) [Okamoto, S., Circulation 82 (Suppl.): 428 (1990)]; angiotensin converting enzyme inhibitors [Gottlieb, N., J. Am. Coll. Cardiol. 17 (Suppl. A): 181A (1991)]; and low molecular weight heparin [de Vries, C., Eur. Heart J. 12 (Suppl.): 386 (1991)].

In an attempt to develop better agents for preventing or reducing smooth muscle proliferation and intimal thickening, the use of balloon catheter induced arterial injury in a variety of mammals has been developed as a standard model of vascular injury that will lead to intimal thickening and eventual vascular narrowing. [Chevru, A., Surg. Gynecol. Obstet. 171: 443 (1990); Fishman, J., Lab. Invest. 32: 339 (1975); Haudenschild, C.,

Lab. Invest. 41: 407 (1979); Clowes, A.W., Lab. Invest. 49: 208 (1983); Clowes, A.W., J. Cardiovas. Pharm. 14: S12 (1989); and Ferns, G.A., Science 253: 1129 (1991)]. Many compounds have been evaluated in this standard animal model. The immunosuppressive agent cyclosporin A has been evaluated and has produced conflicting results. Jonasson reported that cyclosporin A caused an inhibition of the intimal proliferative lesion following arterial balloon catheterization in vivo, but did not inhibit smooth muscle cell proliferation in vitro. [Jonasson, L., Proc.Natl. Acad. Sci. 85: 2303 (1988)]. Ferns, however reposed that when de-endothelilized rabbits were treated with cyclosporin A, no significant reduction of intimal proliferation was observed in vivo. Additionally, intimal accumulations of foamy macrophages, together with a number of vacuolated smooth muscle cells in the region adjacent to the internal elastic lamina were observed, indicating that cyclosporin A may modify and enhance lesions that form at the sites of arterial injury. [Ferns, G.A., Circulation 80 (Supp): 184 (1989); Ferns, G., Am. J. Path. 137: 403 (1990)].

Rapamycin, a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus [U.S. Patent 3,929,992] has been shown to prevent the formation of humoral (IgE-like) antibodies in response to an albumin allergic challenge [Martel, R., Can. J. Physiol. Pharm. 55: 48 (1977)], inhibit murine T-cell activation [Staruch, M., FASEB 3: 3411 (1989)], prolong survival time of organ grafts in histoincompatible rodents [Morris, R., Med. Sci. Res. 17: 877 (1989)], and inhibit transplantation rejection in mammals [Calne, R., European Patent Application 401,747]. Rapamycin blocks calcium-dependent, calcium-independent, cytokine-independent and constitutive T and B cell division at the G1-S interface. Rapamycin inhibits gamma-interferon production induced by I1-1 and also inhibits the gamma-interferon induced expression of membrane antigen. [Morris, R.E., Transplantation Rev. 6: 39 (1992)].

## DESCRIPTION OF THE INVENTION

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of rapamycin to said mammal. The administration may be by one or more of the following routes: orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated with rapamycin.

As such, rapamycin is useful in preventing or treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to autoimmune disorders; alloimmune related disorders; infectious disorders including endotoxins and herpes viruses such as cytomegalovirus; metabolic disorders such as atherosclerosis; and vascular injury resulting from hypothermia, hypothermia, and irradiation. Mechanically mediated vascular injury includes, but is not limited to vascular injury caused by catheterization procedures or vascular scraping procedures such as percutaneous transluminal coronary angioplasty; vascular surgery; transplantation surgery; laser treatment; and other invasive procedures which disrupt the integrity of the vascular intima or endothelium.

Preventing includes the prophylactic prevention of hyperproliferative vascular disease in a susceptible mammal and treating includes arresting the development, and retarding the progression of hyperproliferative vascular disease in a susceptible mammal.

This invention also provides a method of using a combination of rapamycin and mycophenolic acid for the same utilities described above. Mycophenolic acid, an antiproliferative antimetabolite, inhibits inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, enzymes in the de novo purine biosynthetic pathway. This results in an inhibition of DNA synthesis which causes an accumulation of cells at the G1-S interface. Other combinations containing rapamycin that are useful for preventing or treating hyperproliferative vascular disease will be apparent to one skilled in the art. These include, but are not limited to, using rapamycin in combination with other antiproliferative antimetabolites.

Accordingly this invention provides a product containing rapamycin and an antiproliferative antimetabolite such as mycophenolic acid as a combined preparation for simultaneous, separate or sequential use in preventing or treating hyperproliferative vascular disease. In a further respect this invention provides a pharmaceutical composition comprising rapamycin, an antiproliferative antimetabolite such as mycophenolic acid and a pharmaceutically acceptable carrier.

The effect of rapamycin on hyperproliferative vascular disease was established in an in vitro and an in vivo standard pharmacological test procedure that emulates the hyperproliferative effects observed in mammals that are undergoing intimal smooth muscle proliferation and are therefore developing restenosis. Cyclosporin A was also evaluated in these test procedures for the purpose of comparison. The combination of rapamycin and mycophenolic acid was evaluated in the in vivo test procedure. The procedures and the results obtained are described below

Rapamycin and cyclosporin A were evaluated in an in vitro standard pharmacological test procedure which emulates the intimal smooth muscle cell proliferation observed following vascular injury. Results were obtained by measuring DNA and protein synthesis in rat smooth muscle cells that have been stimulated with a growth factor such as fetal calf serum or a hypertrophic mitogen, such as angiotensin II. The following briefly describes the procedure that was used. Rat smooth muscle cells were maintained in a 1:1 mixture of defined Eagle's medium (DEM) and Ham's F12 medium with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 mg/mL) and 25 mL Hepes at pH 7.4. Cells were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ with media changes every 2-3 days. Each compound tested was diluted with an appropriate vehicle to obtain a 1 mM stock solution. Ethanol was used as the vehicle for rapamycin and 20% tween 80 in ethanol was the vehicle for cyclosporin A. Test concentrations of drug were obtained by diluting appropriate concentrations of stock solution with serum free media. The smooth muscle cell culture was maintained in a defined serum free media containing 1:1 DEM and Ham's F12 medium, insulin ($5 \times 10^{-7}$ M), transferrin (5 µg/mL), and ascorbate (0.2 mM) for 72 hours before testing in a multi-well plate. After the 72 hour period, an appropriate quantity of stock solution containing either rapamycin or cyclosporin A was added to the smooth muscle cell culture and media mixture. After a 24 hours the appropriate growth factor was added. For the measurement of DNA synthesis, $^3$H-thymidine was added at 12 hours after the growth factor was added, and the cells were harvested at 36 hours. For the measurement of protein synthesis, $^3$H-leucine was added at 14 hours after the growth factor was added, and the cells were harvested at 18 hours. The amount of incorporated radioactive label was measured on a scintillation counter.

The following table shows the results obtained for rapamycin on DNA and protein synthesis in smooth muscle cells that were stimulated with 10% fetal calf serum, as measured by incorporation of tritiated thymidine or leucine into smooth muscle cells. The amount of yitiated label incorporated by the smooth muscle cells that were treated with media only was normalized to 100%, and the results for cells treated with fetal calf serum or fetal calf serum plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON DNA AND PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH FETAL CALF SERUM*

| | $^3$H-Thymidine Incorporation (% of Media) | $^3$H-Leucine Incorporation (% of Media) |
|---|---|---|
| Media | 100% | 100% |
| FCS | 495% | 174% |
| 1000 nM RAP + FCS | 136% | 95% |
| 100 nM RAP + FCS | 172% | 91% |
| 10 nM RAP + FCS | 204% | 74% |
| 1 nM RAP + FCS | 403% | 106% |

* Abbreviations: RAP = rapamycin; Media = defined serum free media; and FCS = 10% fetal calf serum.

The following table shows the results obtained for rapamycin on protein synthesis in smooth muscle cells that were stimulated with $10^{-6}$ nM angiotensin II, as measured by incorporation of yitiated leucine into smooth muscle cells. The amount of tritiated label incorporated by the smooth muscle cells that were treated with media only were normalized to 100%, and the results for cells treated with angiotensin or angiotensin plus the test compound are expressed as a percent comparison with the cells treated with media only.

EFFECT OF RAPAMYCIN ON PROTEIN SYNTHESIS IN SMOOTH CELLS STIMULATED WITH ANGIOTENSIN II*

| | $^3$H-Leucine Incorporation (% of Media) |
|---|---|
| Media | 100% |
| ANG | 159% |
| 1000 nM RAP + ANG | 53% |
| 100 nM RAP + ANG | 57% |
| 10 nM RAP + ANG | 61% |
| 1 nM RAP + ANG | 60% |

* Abbreviations: RAP = rapamycin; Media = defined serum free media; and ANG = $10^{-6}$ nM angiotensin II.

The results of the standard in vitro, test procedure showed that rapamycin had a pronounced antiproliferative effect in the presence of FCS and an anti-hypertrophic effect in the presence of angiotensin II. Following vascular injury, DNA and protein synthesis of smooth muscle cells are necessary for the development of restenosis to occur. These results showed that rapamycin inhibited both DNA and protein synthesis in stimulated smooth muscle cells. An antiproliferative effect was also observed with cyclosporin A; however, at 1000 nM, cyclosproin A was cytotoxic and not merely cytostatic. At 1000 nM, cyclosporin A caused lysis of the smooth muscle cells as evidenced by the presence of lactic acid dehydrogenase in the supernatent of the cell culture. Similar toxicity to smooth muscle cells was not observed for rapamycin.

Rapamycin, rapamycin plus mycophenolic acid, and cyclosporin A were evaluated in an in vivo standard pharmacological test procedure that emulates the vascular injury suffered and restenosis that develops following percutaneous transluminal coronary angioplasty in humans. The ability of a test compound to inhibit restenosis was determined by comparing intimal thickening in mammals treated with test compound following balloon catheterization versus intimal thickening in untreated control mammals after the same test procedure. [Chevru, A., Surg. Gynecol. Obstet. 171: 443 (1990); Fishman, J., Lab. Invest. 32: 339 (1975); Haudenschild, C., Lab. Invest. 41: 407 (1979); Clowes, A.W., Lab. Invest. 49: 208 (1983); Clowes, A.W., J. Cardiovas. Pharm. 14: S12 (1989); and Ferns, G.A., Science 253: 1129 (1991)]. The following briefly describes the procedure that was used. The left carotid arteries of male Sprague-Dawley rats were injured with an inflated 2Fr balloon catheter. During a 14 day postoperative period, these rats were divided into groups and treated daily with rapamycin (1.5 mg/kg; i.p.), rapamycin plus mycophenolic acid (1.5 mg/kg; i.p. + 40 mg/kg; p.o.), or cyclosporin A (3 mg/kg; i.p.). Additionally, one group each also received rapamycin (6 mg/kg/day; i.p.) or cyclosporin A (40 mg/kg/day; i.p.) for two days postoperatively, and then received no treatment for the next 12 days. An untreated group was used an injured control to establish the amount of intimal growth in the absence of treatment. The right carotid was used as an uninjured control in all groups. After the 14-day period, the rats were sacrificed, the carotids removed. The mean areas of the intima and blood vessel wall were measured by morphometry. Results are expressed as an intima percent which can be expressed according to the following formula:

$$\frac{\text{area of intima}}{\text{area of vessel}} * 100$$

The following table shows the results that were obtained.

EFFECT OF RAPAMYCIN ON INTIMAL THICKENING IN INJURED CAROTID ARTERIES*

| Test Group | Intima Percent ± S.E. |
|---|---|
| Uninjured Control | 0.00 ± 0.00 |
| Untreated Injured Control | 33.3 ± 19.66 |
| RAP (1.5 mg/kg - 14 days) | 6.78 ± 4.69 |
| RAP (6 mg/kg - 2 days) | 16.56 ± 6.22 |
| RAP + MPA (14 days) | 1.6 ± 3.5 |
| CsA (3 mg/kg - 14 days) | 26.46 ± 27.42 |
| CsA (40 mg/kg - 2 days) | 31.14 ± 20.66 |

* Abbreviations RAP = rapamycin; MPA = mycophenolic acid; and CsA = cyclosporin A.

These results show that treatment with rapamycin (1.5 mg/kg for 14 days) resulted in an 80% decrease in the mean percentage intimal thickening compared with the untreated injured control group. Similarly, treatment with the combination of rapamycin and mycophenolic acid produced almost a complete inhibition of intimal thickening (95% reduction in intimal thickening compared with untreated injured control). Cyclosporin A failed to produce any meaningful reduction in intimal thickening.

The results of the in vitro and in vivo standard test procedures demonstrate that rapamycin and rapamycin in combination with mycophenolic acid are useful in preventing or treating hyperproliferative vascular disease. Specifically, rapamycin is useful in preventing or treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion in a mammal, particularly following either biologically or mechanically mediated vascular injury, or under conditions that would predispose a mammal to suffering such a vascular injury.

Rapamycin was also evaluated in a modification of the in vivo test procedure described above. In the modified test procedure, treatment with rapamycin was stopped on day 14, as above, but the animals were not sacrificed immediately. Intimal thickening was observed when the animals were sacrificed 1, 2, or 4 weeks after treatment had been stopped. Microscopic analysis showed that endothelium regeneration had not occurred during the two week treatment period. Following cessation of treatment with rapamycin intimal proliferation, that was previously suppressed, was able to occur. These results are consistent with the results shown in the table above, in which treatment for 2 days with rapamycin followed by 12 days of no treatment inhibited intimal thickening to a lesser degree than treatment with rapamycin for 14 days. These results are expected, as in the absence on an integral endothelial layer, the intimal smooth muscle cells will proliferate. It has been shown that intimal smooth muscle cell growth does not have an inhibitory effect on normal endothelial regeneration, and that intimal smooth muscle cell proliferation ceases when the endothelial layer is established. [Reidy, M., Lab. invest. 59: 36 (1988); Chevru, A., Surg. Gynecol. Obstet. 171: 443 (1990); Fishman, J., Lab. Invest. 32: 339 (1975); Haudenschild, C., Lab. Invest. 41: 407 (1979)]. As such, treatment with rapamycin or rapamycin in combination with mycophenolic acid should be employed until endothelial healing has occurred.

When rapamycin is employed alone or in combination with mycophenolic acid in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dexyin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid car-

rier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

Rapamycin, alone or in combination with mycophenolic acid, may be administered rectally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. Rapamycin, alone or in combination with mycophenolic acid, may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Rapamycin, alone or in combination with mycophenolic acid can be administered intravascularly or via a vascular stent impregnated with rapamycin, alone or in combination with mycophenolic acid, during balloon catheterization to provide localized effects immediately following injury.

Rapamycin, alone or in combination with mycophenolic acid, may be administered topically as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1 - 5 percent, preferably 2%, of active compound.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily intravenous dosages of rapamycin, when administered as the sole active compound, would be 0.001 - 25 mg/kg, preferably between 0.005 - 5 mg/kg, and more preferably between 0.01 - 0.5 mg/kg. Projected daily oral dosages of rapamycin, when administered as the sole active compound or in combination with mycophenolic acid, would be 0.005 - 50 mg/kg, preferably between 0.01 - 25 mg/kg, and more preferably between 0.05 - 10 mg/kg. Projected daily intravenous dosages of mycophenolic acid, when used in combination with rapamycin, would be 0.5 - 25 mg/kg and preferrably between 5 - 25 mg/kg. Projected daily oral dosages of mycophenolic acid, when used in combination with rapamycin, would be 1 - 75 mg/kg and preferrably between 10 - 50 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intravascular, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, rapamycin is most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects, and can be administered either as a single unit dose, or if desired, the dosage may be divided into convenient subunits administered at suitable times throughout the day.

## Claims

1. Use of rapamycin in the manufacture of a medicament for use in preventing or treating hyperproliferative vascular disease in a mammal.

2. Use as claimed in Claim 1 in which the medicament is adapted for administration orally, parenterally, intravascularly, intranasally, intrabronchially, transdermally, rectally, or via a vascular stent impregnated

with rapamycin.

3. Use as claimed in Claim 1 or Claim 2 in which the medicament comprises mycophenolic acid for simultaneous separate or sequential administration.

4. Product containing rapamycin and mycophenolic acid as a combined preparation for simultaneous, separate or sequential use in preventing or treating hyperproliferative vascular disease.

5. A use or product according to any one of claims 1 to 4 wherein the hyperproliferative vascular disease is selected from intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion.

6. A use or product according to any one of Claims 1 to 4 wherein the rapamycin is administered concurrent with and/or subsequent to said mammal undergoing a percutaneous transluminal coronary angioplasty procedure.

7. A use or product according to any one of Claims 1 to 4 wherein the hyperproliferative vascular disease is restenosis.

8. A use or product according to any one of Claims 1 to 4 wherein the rapamycin is administered concurrent with and/or subsequent to said mammal sustaining a biologically or mechanically mediated vascular injury.

9. A pharmaceutical composition comprising rapamycin, mycophenolic acid and a pharmaceutically acceptable carrier.

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# EUROPEAN SEARCH REPORT

Application Number

EP 93 30 0052



## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| X | TRANSPLANTATION PROCEEDINGS<br>vol. 23, no. 6, December 1991,<br>pages 2833 - 2836<br>Y. AKSELBAND 'RAPAMYCIN INHIBITS SPONTANEOUS AND FIBROBLAST GROWTH FACTOR â-STIMULATED PROLIFERATION OF ENDOTHELIAL CELLS AND FIBROBLASTS' | 1,5-8 | A61K31/71 |
| Y | * the whole document * | 1-9 | |
| X | THE LANCET<br>vol. 338, no. 8778, 23 November 1991,<br>pages 1297 - 1298<br>B.M. MEISER ET AL. 'EFFECTS OF CYCLOSPORIN, FK506, AND RAPAMYCIN ON GRAFT-VESSEL DISEASE' | 1,2,5-8 | |
| Y | * the whole document * | 1-9 | |
| X | CYTOKINE<br>vol. 3, no. 5, September 1991,<br>page 472<br>J. WOO ET AL. 'INFLUENCE OF IMMUNOSUPPRESSIVE MACROLIDES AND MYCOPHENOLIC ACID ON OKT3- AND IL-2-INDUCED HUMAN LYMPHOCYTE PROLIFERATION AND IL-2R EXPRESSION' | 4,9 | TECHNICAL FIELDS SEARCHED (Int. Cl.5)<br>A61K |
| Y | * abstract * | 3 | |
| P,X | FASEB J.<br>vol. 6, no. 4, 1992,<br>page A940<br>C. GREGORY ET AL. 'USE OF ANTIPROLIFERATIVE AGENTS FOR THE TREATMENT OF OCCLUSIVE VASCULAR DISEASE'<br>* abstract * | 1-9 | |

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The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 01 APRIL 1993 | KRAUTBAUER B. |

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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|---|---|---|---|
| D,Y | PROC. NATL. ACAD. SCI. USA<br>vol. 85, April 1988,<br>pages 2303 - 2306<br>L. JONASSON ET AL. 'CYCLOSPORIN A INHIBITS SMOOTH MUSCLE PROLIFERATION IN THE VASCULAR RESPONSE TO INJURY'<br>* the whole document * | 1-3,5-8 | |
| Y | TRANSPLANTATION PROCEEDINGS<br>vol. 23, no. 1, February 1991,<br>pages 507 - 508<br>S.M. STEPKOWSKI ET AL. 'PROLONGATION BY RAPAMYCIN OF HEART, KIDNEY, PANCREAS, AND SMALL BOWEL ALLOGRAFT SURVIVAL IN RATS'<br>* the whole document * | 1-9 | |
| Y | US-A-5 078 999 (L.M. WARNER ET AL.)<br>7 January 1992<br>* claim 1 * | 2,4,9 | |
| Y | TRANSPLANTATION<br>vol. 50, no. 4, October 1990,<br>pages 554 - 558<br>D.V. CRAMER ET AL. 'CARDIAC TRANSPLANTATION IN THE RAT'<br>* page 554 *<br>* page 557 * | 1,2,5-8 | TECHNICAL FIELDS SEARCHED (Int. Cl.5) |
| Y | IMMUNOLOGY<br>vol. 72, no. 4, April 1991,<br>pages 544 - 549<br>J.E. KAY ET AL. 'INHIBITION OF T AND B LYMPHOCYTE PROLIFERATION BY RAPAMYCIN'<br>* abstract * | 1,2,5-8 | |

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& : member of the same patent family, corresponding document

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## DOCUMENTS CONSIDERED TO BE RELEVANT

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|---|---|---|---|
| Y | SCAND. J. IMMUNOL.<br>vol. 33, no. 2, February 1991,<br>pages 161 - 173<br>E.M. EUGUI ET AL 'LYMPHOCYTE-SELECTIVE CYTOSTATIC AND IMMUNOSUPPRESSIVE EFFECTS OF MYCOPHENOLIC ACID IN VITRO: ROLE OF DEOXYGUANOSINE NUCLEOTIDE DEPLETION'<br>* abstract *<br>* page 171 *<br>--- | 3,4,9 | |

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

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|---|---|---|
| THE HAGUE | 01 APRIL 1993 | KRAUTBAUER B. |

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